AWARD NUMBER: W81XWH-14-1-0451

TITLE: Inflammatory Role of Macrophage Xanthine Oxidoreductase in Pulmonary Hypertension: Implications for Novel Therapeutic Approaches

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REPORT DATE: **October 2015**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE

Inflammatory Role of Macrophage Xanthine Oxidoreductase in Pulmonary Hypertension: Implications for Novel Therapeutic Approaches

5a. CONTRACT NUMBE

5b. GRANT NUMBER

W81XWH-14-1-0451

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: In Year 1 of this project, we have made a substantial progress toward the proposed original specific aims (SAs) and the major goal of identifying the role of XOR in macrophage inflammatory activation. Both reactive oxygen species (ROS) and macrophages are involved in the pathogenesis of pulmonary hypertension. Xanthine oxidoreductase (XOR) is an ROS generator that plays a central role in inflammation that may contribute to pulmonary vascular inflammation. To identify the role of macrophage specific XOR, we developed a conditional cell specific XOR knockout in mice. During year 1, we characterized this novel previously unavailable conditional XOR KO and showed that macrophages from myeloid specific XOR knockout exhibited loss of inflammatory activation and increased expression of anti-inflammatory markers **(SA1a).** Transcriptional profiling demonstrated an unexpected role for XOR in expression of the NLRP3 inflammasome and acquisition of the glycolytic phenotype by inflammatory macrophages. Recently we have obtained preliminary data showing XOR as a critical regulator of mitochondrial function during hypoxia **(SA1b).** These data demonstrate XOR-Uric acid as a novel drugable targets in stress induced lung parenchymal as well as vascular inflammation.

15. SUBJECT TERMS

Xanthine Oxidoreductase, Macrophage, Pulmonary hypertension, Inflammasone, Mitochondrial Repiration

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

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1- Introduction:

Despite new advances in diagnosis and treatment, pulmonary hypertension (PH) remains a devastating and poorly treated disease with high mortality affecting both adult and pediatric patients. While it can arise for unknown idiopathic or genetic reasons, it is frequently associated with chronic lung disease, pulmonary fibrosis, cardiovascular disease, viral infection, obesity, diabetes, and many other disorders. Lack of understanding of disease mechanism has limited therapeutic opportunities for PH. Inflammatory mechanisms appear to play a significant role in the pathogenesis of all forms of PH. The mechanisms contributing to the initiation and persistence of inflammation in PH remain unclear. Recruitment of monocytes/macrophages has been implicated in this process. Although the exact contribution of macrophages to hypoxia induced PH are unknown, oxidative stress has been implicated in this process. Xanthine Oxidoreductase (XOR) is a source of both reactive oxygen species (ROS) and uric acid that are involved in many inflammatory lung disease. Macrophage XOR was found to regulate adhesion *in vivo*, contribute to cytokine induced ALI, and promote macrophage inflammatory activation[4]. XOR catalyzes the terminal two steps of purine degradation and is the sole biosynthetic source of uric acid (UA). Recent studies demonstrated that UA is an important intracellular mediator of inflammation and its local production in lung could activate the NALP3 inflammasome with IL-1b production in a model of lung fibrosis[5], mobilize dendritic cells by inducing inflammatory Flt3l during plasmodium infection, and induce T Helper 2 cell immunity as an inflammatory mediator of allergic asthma. The preliminary data show increase in pulmonary macrophage XOR activity, lung UA level, and inflammatory cytokines during the first four weeks of hypoxia exposure in rats. Previous studies identified XOR as a source of arterial ROS only early in hypoxia induced PH without identifying the cellular source. These findings suggest the involvement of XOR dependent pathways beyond ROS (e.g. XOR induced UA production) which could play a role in hypoxia induced pulmonary vascular remodeling. To address these issues, a novel conditional knockout of XOR in mice was developed and used here to investigate the specific role of macrophage specific XOR in hypoxia induced PH. The overall hypothesis is that increase in pulmonary macrophage XOR expression and activity plays a key role in PH associated vascular inflammation and remodeling in part by promoting inflammatory activation of pulmonary macrophages.

2- Keywords:

Pulmonary Hypertension, Xanthine Oxidoreductase, Xanthine Dehydrogenase Reactive Oxygen Species, Acute Lung Inflammation, Uric Acid, Chronic Obstructive Pulmonary Disease, Mononuclear Phagocyte, Monosodium Urate, XOR WT, XOR KO, Wistar Kyoto, Pulmonary Artery Systolic Pressure, mean Pulmonary Artery Pressure, Post Capillary Wedge Pressure, Pulmonary Vascular Resistance.

3- Accomplishments:

- ***What were the major goals and objectives of the project?***

SPECIFIC AIM 1. To determine the contribution of macrophage XOR to pulmonary vascular remodeling in a hypoxia induced model of pulmonary hypertension.

Aim 1a. Determine the phenotype of XOR fl/fl//Lysm-Cre by purification and characterization of lung macrophages. **[Year 1]- 100% completed.**
Aim 1b. Determine the inflammatory role of macrophage XOR in hypoxia induced model of PH. **[Year 1]-75% completed.**

SPECIFIC AIM 2. To determine the potential clinical value of modulating XOR pharmacologically in hypoxic rat model of PH.

Aim 2a. Pharmacological modulation of XOR in hypoxia induced Pulmonary Hypertension in both mice and rats. **[Year 1-2]- 50% completed.**
Aim 2b. Assessment of XOR pharmacologic inhibition on pulmonary vascular remodeling in hypoxia induced PH. **[Year 1-2]-50% completed.**

SPECIFIC AIM 3. To determine if serum Uric Acid (UA) is predictive of clinical outcome in PH patients with Chronic Obstructive Pulmonary Disease (COPD).

Aim 3a. To determine Uric Acid Correlation and Covariance Analysis. **[Year 1-2]- 50% completed.**
Aim 3b. COPD Subset Stratification. **[Year 1-2]-20% completed.**

- ***What was accomplished under these goals?***

SA 1. To determine the contribution of macrophage XOR to pulmonary vascular remodeling in a hypoxia induced model of pulmonary hypertension.

Aim 1a. Determine the phenotype of XOR fl/fl//Lysm-Cre by purification and characterization of lung macrophages.

We hypothesized that myeloid XOR specifically was a critical mediator of inflammation and innate immunity that could reveal potentially novel targets for treatment of lung inflammation and hypoxia induced pulmonary vascular remodeling. Germline knockout of XOR in heterozygous mice (XOR+/-) has led to important insights concerning its role in lactation and mammary gland biology, adipocyte differentiation, and metabolic regulation. However, homozygous germline knockout mice (XOR-/-) exhibit early neonatal lethality and cannot be used for analysis of inflammatory disorders that are routinely performed on adult mice. Therefore, germline XOR knockout has been of limited value for analysis of macrophage specific function *in vivo*. For these reasons we sought to develop conditional cell specific knockouts of XOR using a Cre recombinase/LoxP deletion strategy. Here we describe the use of these mice to identify the function of myeloid specific XOR on the development of lung inflammation in mice. TLR4 signaling has been proposed in hypoxia induced lung inflammation. To focus on this signaling pathway and investigate the differential inflammatory role of XOR in the macrophages we used the LPS treatment both in vitro and in vivo.

Generation of Conditional XOR Knockout Mice
To produce a conditional myeloid specific knockout of XOR, we generated mice carrying LoxP sites flanking exons E3 and E4 as illustrated (Figures 1). XOR activity was unaltered by incorporation of LoxP sites compared to parental C57BL/6 mice (Figures 1), while ectopic expression of Cre recombinase cDNA produced efficient ablation of XOR activity in purified TGE macrophages from $XOR^{fl/fl}$, but not C57BL/6 mice (Figure 1C). To knockout XOR conditionally in myeloid lineage cells, including neutrophils and macrophages, we crossed $XOR^{fl/fl}$ mice with C57BL/6:LysM-Cre mice and selected for $XOR^{fl/fl}$ by brother sister cross of the heterozygotes to create the $XOR^{fl/fl//LysM\text{-}Cre}$ strain. These mice were selected to express LysM-promoter regulated Cre recombinase in heterozygous state and $XOR^{fl/fl}$ in homozygous state as described. Cre recombinase mediated recombination will generate a shift in the XOR reading frame resulting in failure to synthesize more than 42 amino acids of 1,338 present in the mature protein.

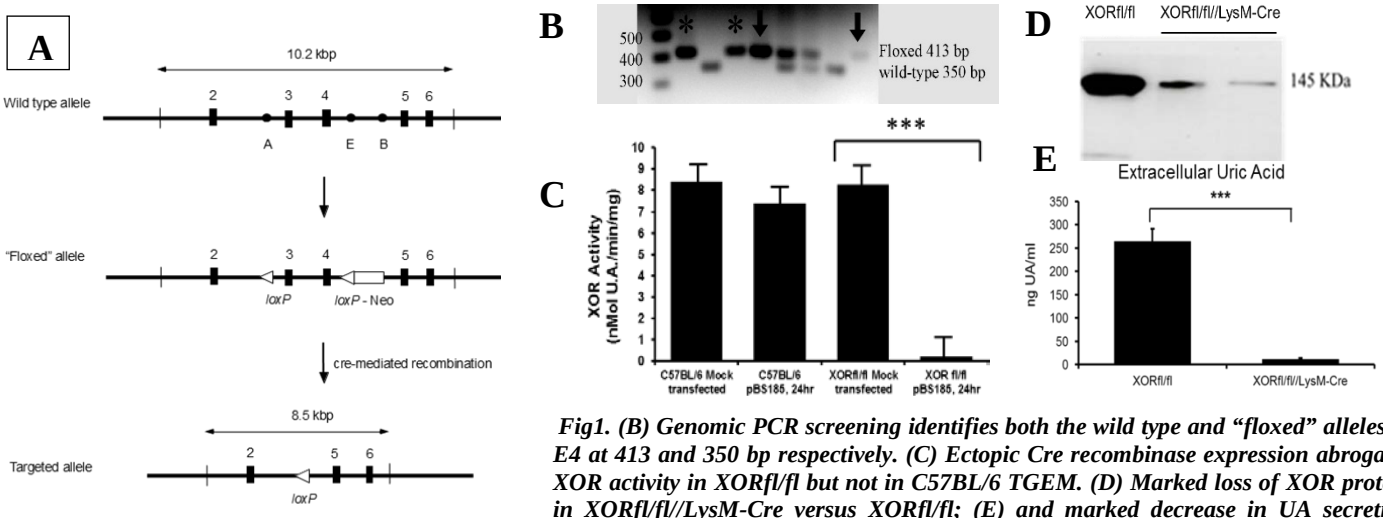


Fig1. (B) Genomic PCR screening identifies both the wild type and "floxed" alleles of E4 at 413 and 350 bp respectively. (C) Ectopic Cre recombinase expression abrogates XOR activity in XORfl/fl but not in C57BL/6 TGEM. (D) Marked loss of XOR protein in XORfl/fl//LysM-Cre versus XORfl/fl: (E) and marked decrease in UA secretion

Mononuclear Phagocyte XOR Activity and Superoxide Generation Were Reduced by Myeloid Specific Ablation

BAL cell XOR activity (Figure 2A) was significantly reduced in $XOR^{fl/fl//LysM\text{-}Cre}$ mice compared to $XOR^{fl/fl}$ mice 48 hrs after LPS insufflation, and we observed that XOR activity from $XOR^{fl/fl}$ BAL cells was largely in Oxidase (O-Form) (>90%) following LPS insufflation but predominantly Dehydrogenase (D-Form) (80%) following saline insufflation (Figure 2B), consistent with XOR as a source of ROS. BAL cell superoxide generated in $XOR^{fl/fl}$ cells was significantly reduced in $XOR^{fl/fl//LysM\text{-}Cre}$ cells following LPS insufflation (Figure 2C), while lung tissue nitrotyrosine staining was reduced to background levels in $XOR^{fl/fl//LysM\text{-}Cre}$ mice compared to XORfl/fl mice (Figure 2D).

To determine which cells in the BAL expressed XOR, cells recovered by lavage from $XOR^{fl/fl}$ mice following LPS insufflation were sorted by FACS into neutrophil (PMN), recruited monocyte/macrophages, and resident alveolar macrophage populations. Very low levels of Allopurinol inhibited XOR activity were detected in the PMN fraction (Figure 2E). While recruited and resident alveolar macrophages together accounted for 94% of the Allopurinol inhibited XOR activity, resident macrophages strongly expressed XOR (Figure 2E). Macrophage survival determined by SRB stain was unaltered by XOR genetic ablation (Figure 2F).

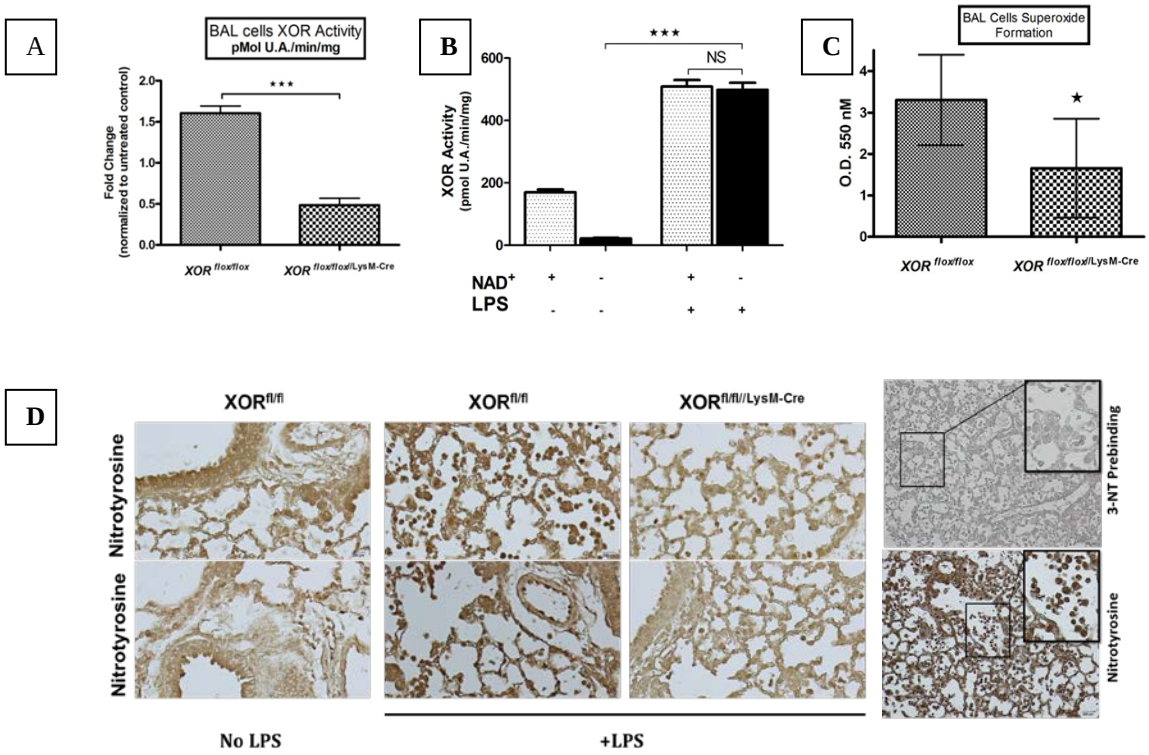


Fig 2

Macrophage XOR Contributes To LPS Induced Inflammasome Expression

As shown by microarray analysis, we observed significant decrease in NLRP3, IL-1β, and TNFα mRNA in $XOR^{fl/fl//LysM\text{-}Cre}$ mice compared to $XOR^{fl/fl}$ mice when whole lung RNA from saline or LPS insufflated mice was screened by quantitative RT-PCR analysis (Figure 3A). We observed no significant effect of XOR knockout on lung IL-18 expression (not shown). Macrophages purified from the BAL of LPS insufflated $XOR^{fl/fl}$ and $XOR^{fl/fl//LysM\text{-}Cre}$ mice also revealed significantly decreased mRNA expression for NLRP3 and IL-1β. Decrease in mRNA for IL-18 followed a similar trend but did not reach statistical significance (Figure 3B). Both NLRP3 and IL-1β protein levels were also greatly reduced in the purified macrophages from $XOR^{fl/fl//LysM\ Cre}$ mice compared to $XOR^{fl/fl}$ mice (Figure 3C). Inflammatory cytokine expression in the cell free BALF was analyzed by ELISA and western blot which demonstrated significantly decreased IL-1β and IL-18 secretion consistent with the reduced expression of IL-1β mRNA in the BAL macrophages of LPS insufflated $XOR^{fl/fl//LysM\text{-}Cre}$ mice (Figure 3D). We also observed marked reduction in pro-caspase-1 and mature p10/p20 caspase-1 proteins in both $XOR^{fl/fl//LysM\text{-}Cre}$ lung tissues (Figure S3) and in whole cell lysates of macrophages purified from the BAL of LPS insufflated mice (Figure 3E).

To determine if XOR derived ROS mediated expression of inflammasome components, purified BAL macrophages were cultured in the presence of the ROS scavenger N-acetylcysteine (NAC) or VAS2870, a selective inhibitor of NADPH oxidase activity and ROS generation. After 24 hrs cells were harvested and analyzed by qRT-PCR for expression of Nlrp3 and IL-1β. While VAS2870 had no significant effect on Nlrp3 or IL-1β expression, both NAC and XOR knockout significantly reduced expression of both Nlrp3 and IL-1β (Figure 3F). The combination of NAC and the XOR knockout revealed a small contribution to LPS induced Nlrp3 and IL-1β that was not derived from XOR generated ROS alone. Nonetheless, these data confirm the principal role of XOR derived ROS in the expression of LPS induced Nlrp3 and IL-1β, but do not support a primary role for the NADPH oxidase.

Fig 3

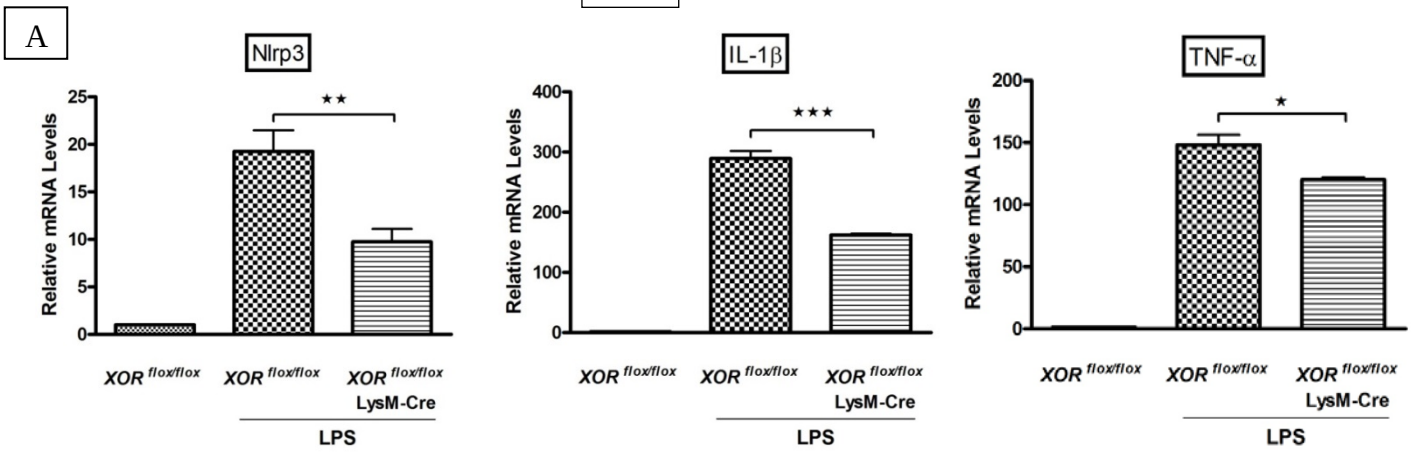
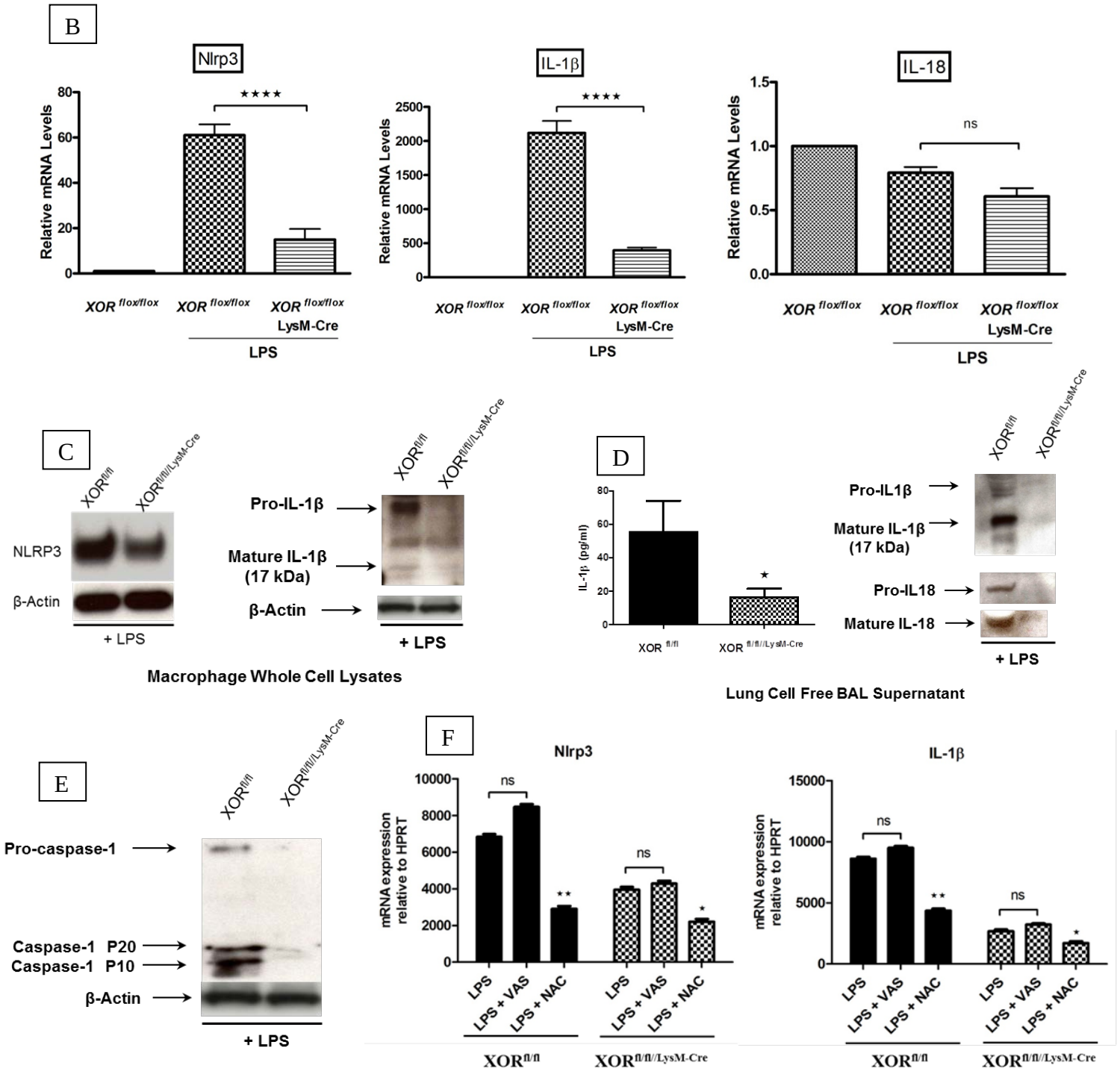


Fig 3

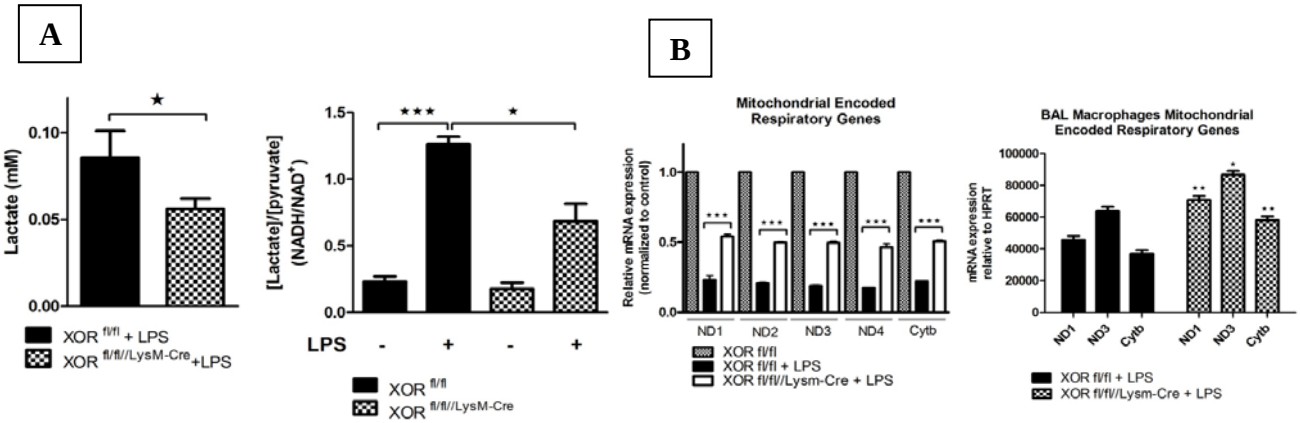


XOR Contributes To The LPS Induced Glycolytic Shift

To verify the effect of XOR ablation on mitochondrial OXPHOS, we measured levels of lactate and pyruvate in purified BMDM derived from $XOR^{fl/fl}$ and $XOR^{fl/fl//LysM\text{-}Cre}$ mice 24 hrs after LPS treatment *in vitro*. Significant reduction in lactate generation by $XOR^{fl/fl//LysM\text{-}Cre}$ macrophages was observed compared to $XOR^{fl/fl}$ macrophages (Figure 4A). The effect of XOR knockout on expression of mitochondrial OXPHOS genes was verified by qRT-PCR of whole lung RNA obtained from saline or

LPS insufflated mice (Figure 4B). LPS markedly suppressed expression of complex I genes (ND1, ND2, ND3, ND4) and Cytochrome b in XOR$^{fl/fl}$ mice, and this effect was significantly reversed in XOR$^{fl/fl//LysM\text{-}Cre}$ mice (Figure 4B).

Fig 4

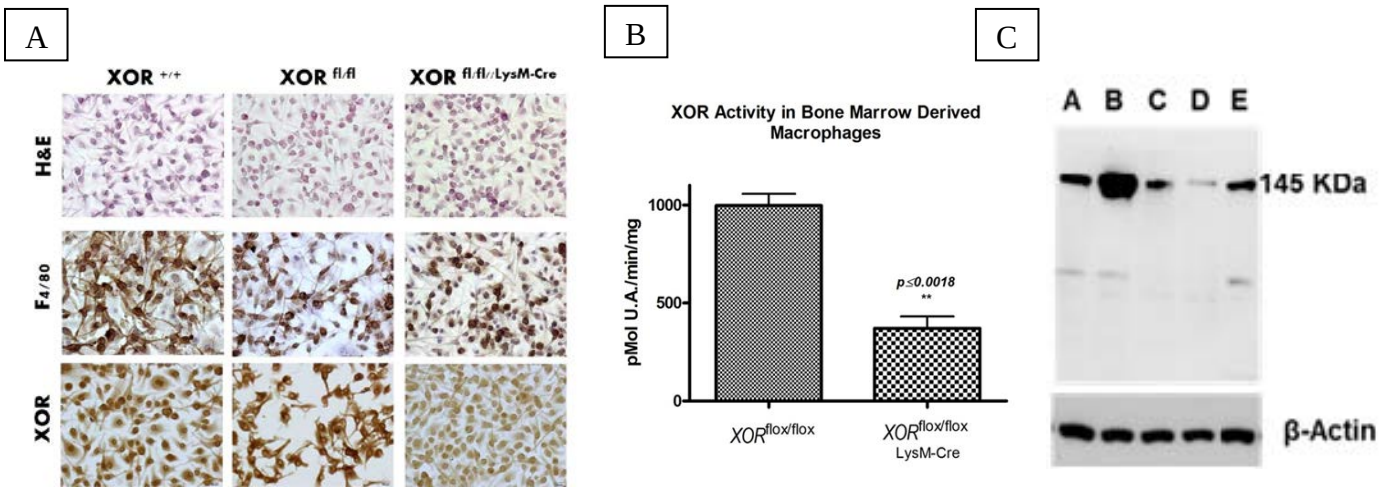


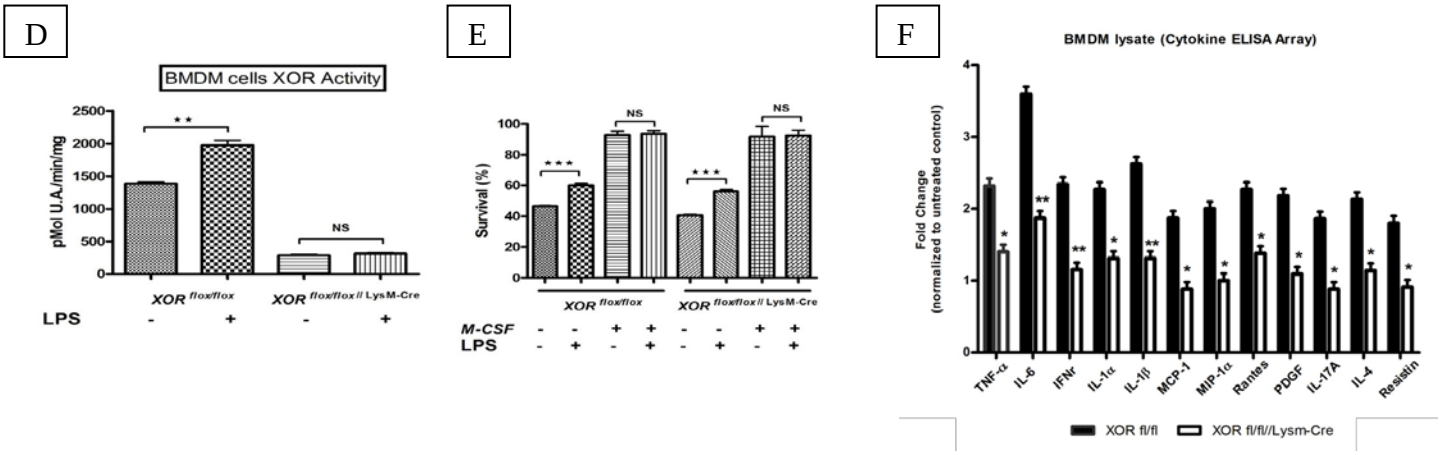
Myeloid Specific XOR Knockout *In vivo* Reduces Activation of BMDM and TGEM

XOR$^{fl/fl}$ and XOR$^{fl/fl//LysM\text{-}Cre}$ were contrasted to determine the contribution of XOR to activation of TGEM and BMDM. BMDM exhibited >90% positive staining for the macrophage marker F4/80 (Figure 5A), and >95% viability following M-CSF differentiation (Figure 5E). XOR activity and protein level were reduced by >70% in BMDM and TGEM from XOR$^{fl/fl//LysM\text{-}Cre}$ mice compared to XOR$^{fl/fl}$ littermates (Figure 5B,C). While F4/80 immunoreactivity (Figure 5A) and cell survival were unaffected by XOR knockout in XOR$^{fl/fl//LysM\text{-}Cre}$ cells (Figure 5E), XOR immunoreactivity was reduced to near background in XOR$^{fl/fl//LysM\text{-}Cre}$ compared to XOR$^{fl/fl}$ BMDM (Figure 5A).

Treatment of BMDM with 100 ng/ml E. coli LPS for 24 hrs increased XOR activity, and both uninduced and LPS induced XOR activity was blocked by XOR knockout in XOR$^{fl/fl//LysM\text{-}Cre}$ mice (Figure 5D). LPS treatment did not reduce survival of M-CSF differentiated BMDM in either XOR$^{fl/fl}$ or XOR$^{fl/fl//LysM\text{-}Cre}$ cells (Figure 5E). The inflammatory marker Galectin-3 showed marked stimulation by LPS in XOR$^{fl/fl}$ derived BMDM that was reduced to control levels in XOR$^{fl/fl//LysM\text{-}Cre}$ cells (Figure 5G). We observed between 2 and 4 fold increase in several inflammatory cytokines in BMDM from XOR$^{fl/fl}$ mice that was significantly reduced (TNFα, IL-6, IL-1α, IL-1β, RANTES) or blocked completely in XOR$^{fl/fl//LysM\text{-}Cre}$ cells after LPS treatment (MCP-1, MIP-1α, IL-17A, Resistin) (Figure 5F).

Fig 5





Aim 1b. Determine the inflammatory role of macrophage XOR in hypoxia induced model of PH. **[Year 1]-75% completed.**

Elevated expression of XOR is associated with accumulation of macrophages in perivascular areas in rat and calf models of hypoxia-induced pulmonary hypertension. Cryosections of rat lung pulmonary arteries were double-labeled with antibodies against XOR (red) and macrophage markers (green, - ED1 for rat, and CD68 for calf). Cell nuclei are counterstained with DAPI (blue) **(Figure 6)**. M = vascular media; Adv. – perivascular adventitia. Scale bar = 100 um.

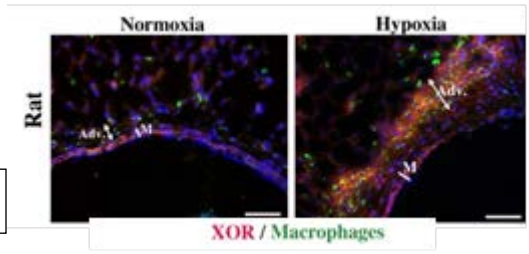
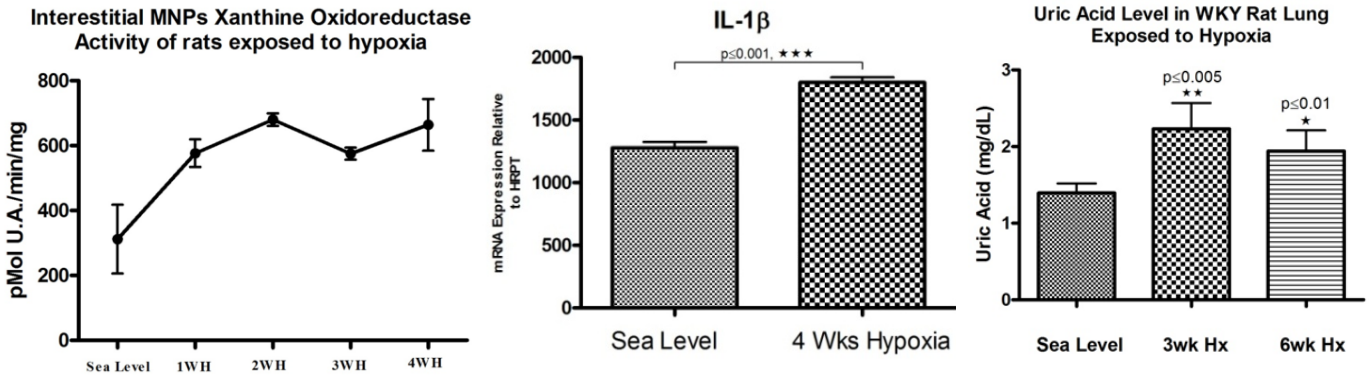


Fig 6

Elevated activity of XOR is associated with increase in lung uric acid level and IL-1β expression in pulmonary macrophages. Wistar Kyoto rats (WKY) were exposed to hypobaric hypoxia (15,000 feet) for 1, 2, 3 and 4 weeks. Interstitial lung (IL) MNPs from sea level age matched control rats and the hypoxic ones were isolated. Total XOR activity was determined. RNA was isolated from the IL MNPs and IL-1β transcripts were measured by semi-quantitative RT-PCR. Total lung uric acid level was quantified according to the methodology explained in the experimental plans **(Figure 7)**.

Fig 7



Hypoxia-induced pulmonary hypertension (in rat models) is associated with augmented perivascular fibrosis (deposition of extracellular matrix proteins, cellular fibronectin and tenascin-C

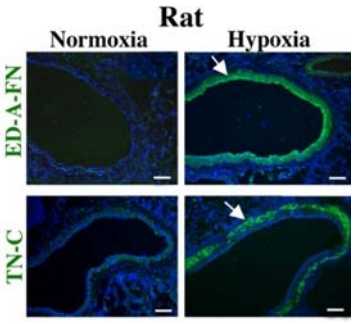


Fig 8

(arrows). Cryosections of rat and calf lung pulmonary arteries were stained (green) with antibodies against markers of fibrosis (cellular, - isoform ED-A, - fibronectin (ED-A-FN) and tenascin-C (TN-C) **(Figure 8)**. Scale bar = 100 um.

Myeloid cell specific LysM-CRE Mediated XOR Knockout. To knockout XOR conditionally in myeloid lineage cells, including macrophages, we crossed C57BL/6J:XORfl/fl mice with C57BL/6J:LysM-CRE mice to create the C57BL/6J:LysM-CRE//XORfl/fl strain. These mice were selected to express LysM regulated CRE recombinase in heterozygous state and XORfl/fl in homozygous state. **$XOR^{fl/fl}$//$Lysm^{Cre-}$ mice exposed to chronic hypoxia demonstrate augmented pulmonary perivascular fibrosis and cardiac hypertrophy, whereas $XOR^{fl/fl}$//$Lysm^{Cre+}$ mice are protected (demonstrate attenuated fibrosis in pulmonary perivascular adventitia and protected heart).** Formalin-fixed paraffin-embedded sections of murine lungs were processed for Pentachrome histological staining. Yellow color defines collagen accumulation in perivascular adventitia (arrow) **(Figure 9)**. Adv. = pulmonary artery adventitia. Scale bar = 50 um.

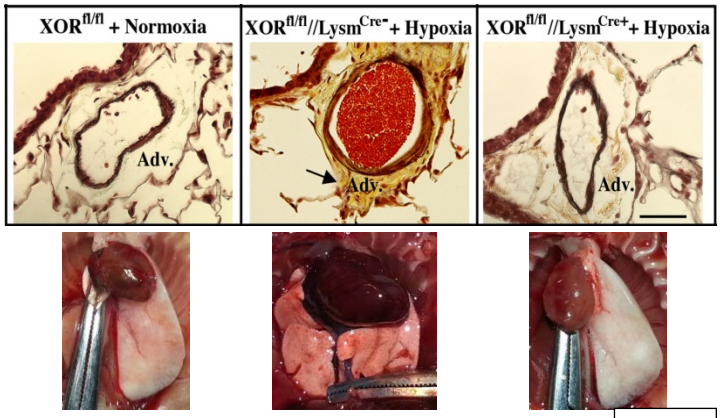


Fig 9

SA 2. To determine the potential clinical value of modulating XOR pharmacologically in hypoxic rat model of PH.

Aim 2a. Pharmacological modulation of XOR in hypoxia induced Pulmonary Hypertension in both mice and rats.
Aim 2b. Assessment of XOR pharmacologic inhibition on pulmonary vascular remodeling in hypoxia induced PH.

Pre-treatment with specific XOR inhibitor (y-700) attenuates pulmonary artery remodeling in rat model of hypoxia-induced pulmonary hypertension. 5 um Cryosections of rat lung processed for Pentachrome histological staining **(Figure 10)**. Yellow color defines collagen accumulation in perivascular adventitia.

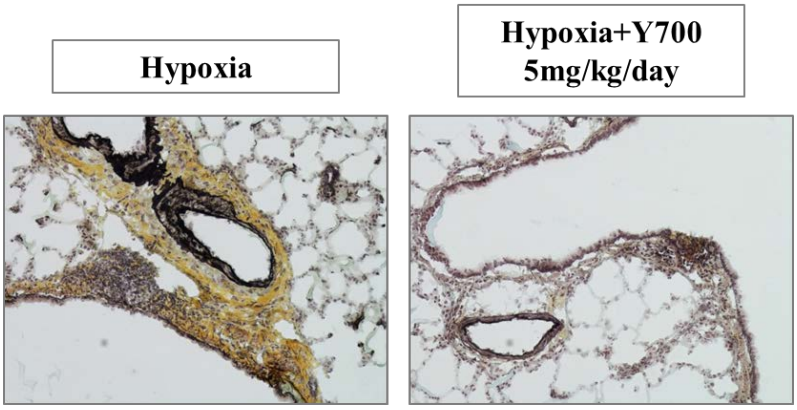


Fig 10

Pre-treatment with specific XOR inhibitor (y-700) improves pulmonary artery hemodynamic parameters in rat model of hypoxia-induced pulmonary hypertension. (Figure 11).

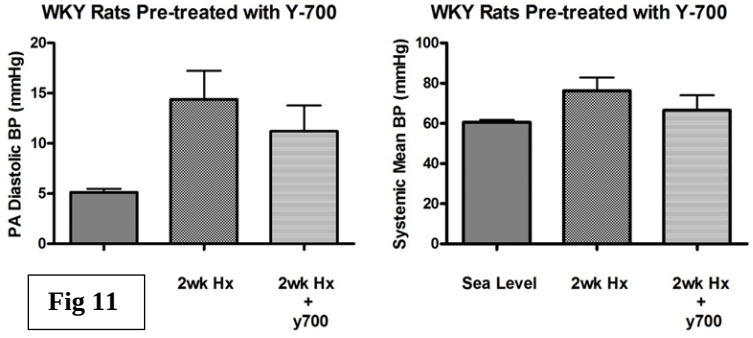


Fig 11

SA 3. To determine if serum Uric Acid (UA) is predictive of clinical outcome in PH patients with Chronic Obstructive Pulmonary Disease (COPD).

Aim 3a. To determine Uric Acid Correlation and Covariance Analysis.
Aim 3b. COPD Subset Stratification.

We have used both the archived retrospective un-identified human patient data from both Denver VA and University of Colorado Denver Hospital. We have been successful to collect retrospective data on 35 patients who were diagnosed with PH and COPD. All the lab and clinical including the imaging will be analyzed according to our approved EXEMPT COMIRB application.

- ***What opportunities for training and professional development did the project provide?***

We were able to hire Ms. Angela Fei, freshman at Berkley, to join us as a summer student through the University of Colorado Summer Student Fellowship Program. Her contribution to the project is acknowledged in the manuscript in revision for the publication in "Cell Reports".

- ***How were the results disseminated to communities of interest?***

Nothing to report.

- ***What do you plan to do during the next reporting period to accomplish the goals and objectives?***

As described in the section: "Changes/Problems", we need to add a new transgenic mouse line (XOR fl/fl//Csfr1-Cre) which we have generated as a new cross with our novel XOR floxed founders. We will use this new line both in our SA1 and SA2 after obtaining the approval on our amendment application with ACURO.

We will continue to collect 15 more retrospective patient data (PH with COPD) and will analyze those data and will compare those to the control COPD patients.

4- IMPACT:

- ***What was the impact on the development of the principal discipline(s) of the project?***

Xanthine oxidoreductase (XOR) plays a central role in inflammation, innate immunity, and associated disorders and it could comprise an important therapeutic target for treatment of ALI/ARDS. To identify the inflammatory role of macrophage specific XOR on the development of lung injury *in vivo*, we developed a conditional cell specific XOR knockout in mice that has never before been achieved. Macrophages from myeloid specific XOR knockouts exhibited loss of inflammatory activation and increased expression of anti-inflammatory markers. Transcriptional profiling of LPS insufflated mice demonstrated an unexpected role for XOR in expression of the NLRP3 inflammasome and acquisition of the glycolytic phenotype by inflammatory macrophages. These results identify XOR as an unexpected physiological link between macrophage inflammatory activation and mitochondrial respiration.

- ***What was the impact on other disciplines?***

Nothing to report.

- ***What was the impact on technology transfer?***

There is an MTA filed with the University of Colorado Transfer Office on the transfer of the novel XOR KO mice to interested investigators/industry after the phenotype and characterization of the mice is complete and published.

- ***What was the impact on society beyond science and technology?***

Nothing to report.

5- CHANGES/PROBLEMS:

- ***Changes in approach and reasons for change.***

To characterize the specific role of macrophage XOR in hypoxia induced lung injury we propose to use a new transgenic mouse line in which XOR is knockout of macrophages as well as circulating monocytes (XOR$^{fl/fl//Csf1r\text{-}Cre)}$. Our current results from XOR $^{fl/fl//LysmCre}$ mice show that XOR is efficiently knocked out in lung resident macrophages and not in circulating monocytes. Our data also confirm our previous data of low XOR activity in neutrophils. We will use our new conditional knockout mouse strains developed by us by crossing mice carry a homozygous Csf1r-Cre expressing transgene that has been placed under the regulation of the Csf-Receptor promoter that exhibits very high, but not exclusive, expression in macrophages and determine the effect on hypoxia induced pulmonary vascular inflammation.

- ***Actual or anticipated problems or delays and actions or plans to resolve them.***

We still need to add 15 more patients' retrospective data to fulfill the requirements proposed in aim 3. We are using the archived retrospective un-identified human patient data from both Denver VA and University of Colorado Denver Hospital. We will try to find our proposed number from the sites originally proposed in our application. We will add more sites to find the required clinical data to complete our proposed study in aim 3.

- ***Changes that had a significant impact on expenditures.***

Not Applicable.

- ***Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.***

Ass proposed above and to achieve our goals as proposed in our original application we proposed to add a new transgenic line. We will seek the ACURO approval for this new change in the use of vertebrate animals.

6- PRODUCTS:

In Year 1 of the project we have one manuscripts in revision for publication in the prestigious Cell Press journal: "Cell Reports" reflecting progress on the project (and in all cases explicitly acknowledging grant support from the DoD/CDMRP/W81XWH-14-1-0451) as follows, with relevant SA's noted in **bold**:

1- Mehdi A. Fini, Jenifer A. Monks, Min Li, Maria Frid, Steven C. Pugliese, Donna Bratton, William Janssen, Richard Scarpulla, Michael Karin, Kurt R. Stenmark, and Richard M. Wright. Macrophage Xanthine Oxidoreductase Mediates LPS Induced Lung Inflammatory Injury In Mice Through Activation Of The Nlrp3 Inflammasome and Mitochondrial OXPHOS. Cell Reports, in revision, 2015 **(SA. 1a).**

Presentations (given by PI; acknowledging DoD/CDMRP/W81XWH-14-1-0451) included:

Fini MA, Vaitaitia G, Wagner D, Stenmark KR. Xanthine Oxidoreductase-Uric Acid Induce Novel Th40 Cells Expansion and Activation in Hypoxic Model of Pulmonary Hypertension. ATS International Conference; 2015 May 06; Denver, Colorado, USA. AJRCCM.

Fini MA, Gu S, McNally A, Burnham EL. Alcohol Use Disorders Are Associated with Differential Expression of Xanthine Oxidoreductase in Alveolar Macrophages. ATS International Conference; 2015 May 06; Denver, Colorado, USA. AJRCCM.

Fini MA, McNally A, Standiford TJ, Moss M, Burnham EL. Bronchoalveolar Lavage Uric Acid Is Elevated in ARDS and Associated With Early Ventilator Parameters. ATS International Conference; 2015 May 06; Denver, Colorado, USA. AJRCCM.

Funding Applied for Based on the Work Supported by this Award includes:

DoD (CDMRP) Investigator Initiated Research Award in response to W81XWH-15-PRMRP-IIRA, "***Fiscal Year 2015 Department of Defense Peer Reviewed Medical Research Program (PRMRP)***" (new application; Fini – Burnham, Partnering PI options) 2016-2019 "Role of Xanthine Oxidoreductase - Uric Acid Axis as Novel Determinants of Macrophages Mediated Inflammatory Responses in Acute Lung Injury/ARDS " [pending]

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

ACCT	(Multiple Items)	
Row Labels	**Sum of ACTUAL**	
	$	-
FEI, ANGELA CHENLI	$	1,420.83
FINI, MEHDI	$	21,008.31
HANEY, TRACY N	$	1,955.43
HEMMATI, ALIREZA	$	885.00
SCHNEIDER, STEPHANIE ELLYSE	$	2,480.00
STENMARK, KURT R	$	9,148.65
WICK, MARILEE J	$	4,453.62
WRIGHT, RICHARD M	$	4,239.95
Grand Total	**$**	**45,591.79**

Name:	Mehdi A. Fini, MD
Project Role:	Principal Investigator
person month worked:	8.5
Contribution to Project:	As principal investigator and project director, I was involved with all aspects of the proposed research.

Name:	Angela Fei, B.Sc
Project Role:	Undergraduate Student
person month worked:	1.19
Contribution to Project:	Ms. Fei was working as a summer student/fellow and was involved with performing various experimental procedure as well as data analysis.

Name:	Tracey Haney, B.Sc
Project Role:	Veterinarian Technician
person month worked:	0.96
Contribution to Project:	Ms. Haney is involved with helping with mouse breeding and colony management.

Name:	Alireza Hwmmati, B.Sc
Project Role:	Technician
person month worked:	1.19
Contribution to Project:	Mr. Hemmati was involved with microscopy, imaging and data Analysis.

Name:	Stephanie Ellyse Schneider, B.Sc
Project Role:	Technician
person month worked:	0.72
Contribution to Project:	Ms. Schneider has been involved with flow cytometry and data analysis.

Name:	Kurt R. Stenmark, MD
Project Role:	Co-Investigator
person month worked:	0.45
Contribution to Project:	Dr. Stenmark provides pre-clinical and clinical assistance with the research proposed in the specific aims 1 and 2.

Name:	Marilee Week, Ph.D.
Project Role:	Research Associate
person month worked:	3.08
Contribution to Project:	Dr. Week provides assistance with some technical experiments with aim 1 and 2.

Name:	Richard M. Wright, Ph.D.
Project Role:	Co-Investigator
person month worked:	1
Contribution to Project:	Dr. Wright was involved with pre-clinical as well as technical assistance with the proposed experiments in aim 1 and 2.

Name:	Edward Dempsey, MD
Project Role:	Co-Investigator
person month worked:	0.45

Contribution to Project:	Dr. Dempsey provides clinical assistance with experiments proposed in aim 3.
Funding Support:	Compensated through VA.

Name:	Richard Johnson, MD
Project Role:	Co-Investigator
person month worked:	0.45
Contribution to Project:	Dr. Johnson provides pre-clinical and clinical guidance for proposed experiments in aim 2 and 3.
Funding Support:	NIH- DOD and clinical salary support through CU.

8. SPECIAL REPORTING REQUIREMENTS: None